Mentype® **Argus X-12** PCR Amplification Kit

Product description

The Mentype® **Argus X-12** PCR Amplification Kit is a multiplex application for twelve X-chromosomal Short Tandem Repeat (STR) loci that supplements the Mentype® **Argus Y-MH**^{QS} and Mentype® **Argus Y-12**^{QS} test kit for kinship and paternity testing especially in complicated deficiency cases. Furthermore the use of gonosomal STRs for the analysis of DNA traces in the forensic field is strongly rising.

The Mentype® **Argus X-12** contains the primers of **Amelogenin (AM)** for gender-determination, **DXS7132**, **DXS7423**, **DXS8378**, **DXS10074**, **DXS10079**, **DXS10101**, **DXS10103**, **DXS10134**, **DXS10135**, **DXS10146**, **DXS10148** and **HPRTB**. Three markers belong to one of the four coupling groups of the X-chromosome (Fig. 1), so that three markers of each group have to be handled as haplotype for genotyping. The primers are fluorescence-labelled with **6-FAM**, **BTG**, **BTR** and **BTY**.

Primer sequences of the Mentype® **Argus X-12** are different to that of the Mentype® **Argus X-8** in order to avoid allelic overlapping. Furthermore, the changing results in more robustness and sensitivity. Because of the new primer design for the locus **HPRTB** a dinucleotide-deletion (AG) 48-49 bp downstream of the repeat unit (Mertens et al. 1999, Gomes et al. 2007) will not be detected any more so that just the real repeat units will be shown. If necessary point alleles (like 11.2, 12.2), that were detected by the primers of the Mentype® **Argus X-8** or **Argus X-UL,** should be rounded up the next full allele for comparing the results generated with the Mentype® **Argus X-12** primers.

The detection limit of the Mentype® **Argus X-12** PCR Amplification Kit is **100 pg genomic DNA**. The optimal range under standard conditions is **0.2-0.5 ng DNA**. However, internal validations demonstrated reliable results with <0.1 ng DNA.

The Forensic ChrX Research Group initiated the online data base **ChrX-STR.org** (http://www.chrx-str.org) that calculates population-genetic data on basis of X-chromosmal allele frequencies (Szibor et al., 2006). Additionally, all important population-genetic data can be calculated with the GenoProof® software.

The test kit was validated and evaluated using the GeneAmp® 9700 thermal cycler, ABI PRISM® 310 Genetic Analyzer, and ABI PRISM® 3100/3130 Genetic Analyzer.

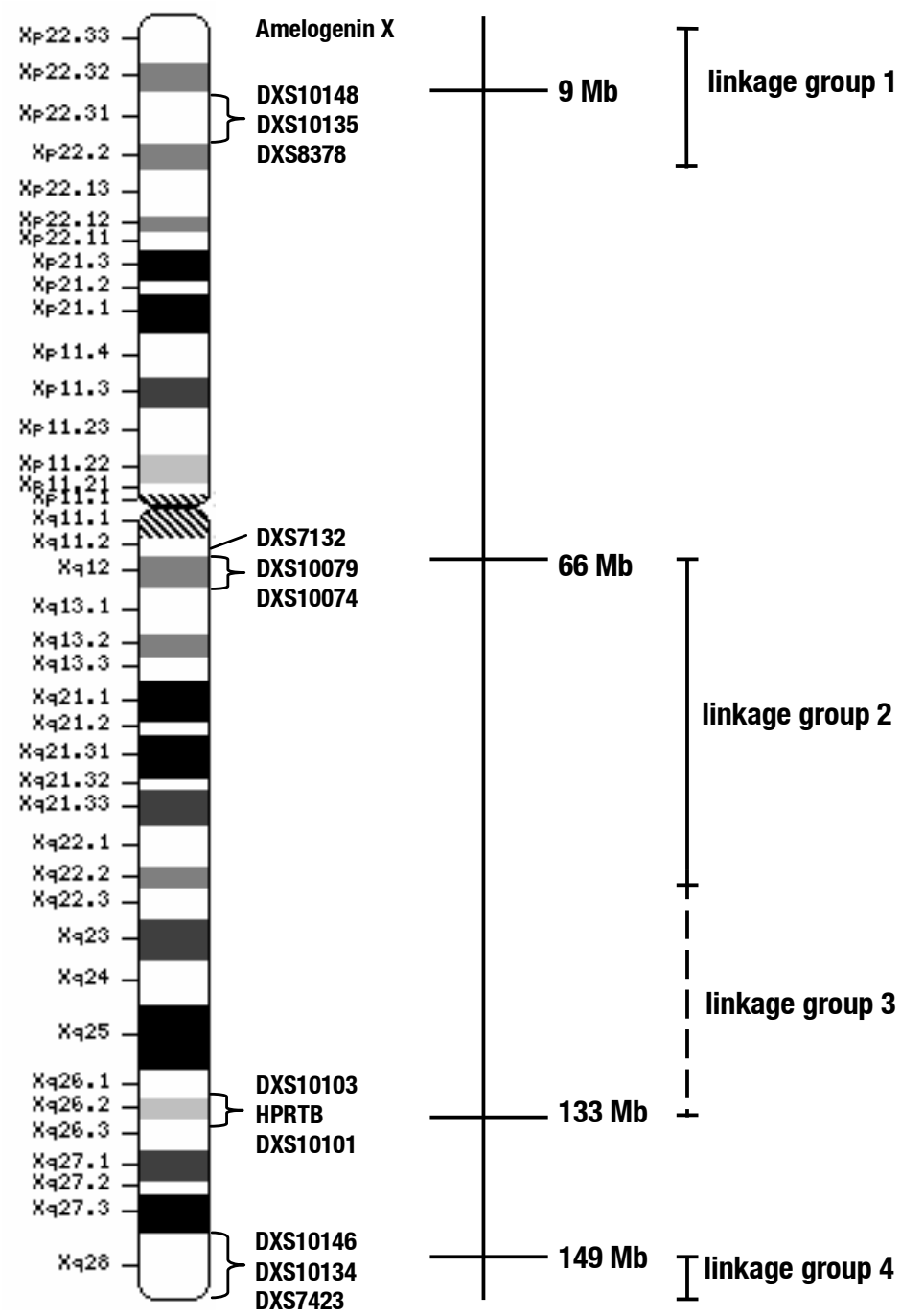

Fig. 1 The ideogram of the X-chromosome describes the physical localisation of the STR loci which can be analysed with Mentype® **Argus X-12.** Distances from the p-telomere are shown in Mb (http://www.ncbi.nlm.nih.gov/genome/guide/human as at 10/2009).

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1. Description of Mentype® Argus X-12

Table 1. Locus-specific information of Mentype® Argus X-12

Locus	GenBank® accession	Repeat motif of the reference allele	Reference allele	Allele range
Amelogenin X	M55418			
Amelogenin Y	M55419			
DXS7132	G08111	$[TCTA]_{13}$	13	8-20
DXS7423	AC109994	$[TCCA]_3$ TCTGTCCT $[TCCA]_{12}$	15	8-19
DXS8378	G08098	$[CTAT]_{12}$	12	7-15
DXS10074	AL356358	$[AAGA]_{14}$	14	4-21
DXS10079	AL049564	$[AGAG]_3$ TGAAAGAG $[AGAA]_{17}$ AGAG $[AGAA]_3$	21	14-25
DXS10101	AC004383	$[AAAG]_3$ GAAAGAAG $[GAAA]_3$ A $[GAAA]_4$ AAGA $[AAAG]_5$ AAAAAGAA $[AAAG]_{13}$ AA	28.2	24-38
DXS10103	BV680555	$[TAGA]_2$ CTGA $[CAGA][TAGA]_{11}[CAGA]_4[TAGA]$	19	15-21
DXS10134	AL034384	$[GAAA]_3$ GAGA $[GAAA]_4$ AA [GAAA] GAGA $[GAAA]_4$ GAGA $[GACAGA]_3$ [GAAA] GTAA $[GAAA]_3$ AAA $[GAAA]_4$ AAA $[GAAA]_{15}$	35	28-46.1
DXS10135	AC003684	$[AAGA]_3$ GAAAG $[GAAA]_{20}$	23	13-39.2
DXS10146	AL034384	$[TTCC]_3$ T $[TTCC]_3$ TTTC CTCCCTTCC [TTCC] [TCCC] TTCTTCTTTC $[TTCC]_2$ TTTCTT $[CTTT]_2$ CTTC $[CTTT]_{10}$ T $[CTTT]_2$	26	24-46.2
DXS10148	AC003684	$[GGAA]_4[AAGA]_{12}[AAAG]_4$ N_8 $[AAGG]_2$	22	13.3-38.1
HPRTB*	M26434	$[AGAT]_{12}$	12	6-19

*[AGAT] is the common repeat structure, for variations see NIST and Szibor et al. 2009.

Table 1 shows the STR loci with their repeat motifs and alleles that are concordant with the International Society for Forensic Genetics (ISFG) guidelines for the use of microsatellite markers (Bär et al., 1997). Allele ranges include all known alleles of the current literature as at 10/2009.

Table 2. Chromosomal mapping of Mentype® Argus X-12

Locus	Chromosomal mapping
Amelogenin X	Xp22.1-22.3
Amelogenin Y	Yp11.2
DXS7132	Xq11.2
DXS7423	Xq28
DXS8378	Xp22.31
DXS10074	Xq12
DXS10079	Xq12
DXS10101	Xq26.2
DXS10103	Xq26.2
DXS10134	Xq28
DXS10135	Xp22.31
DXS10146	Xq28
DXS10148	Xp22.31
HPRTB	Xq26.2

Content

Mentype® **Argus X-12** PCR Amplification Kit (100 reactions)

Nuclease-free water	3.0 mL
Reaction mix **A**	500 µL
Primer mix	250 µL
Control DNA XX28 (2 ng/µL)	10 µL
DNA Size Standard 550 (BTO)	50 µL
Allelic ladder	25 µL

Ordering information

Mentype® **Argus X-12**	25	Reactions	Cat. No.	43-13110-0025
Mentype® **Argus X-12**	100	Reactions	Cat. No.	43-13110-0100
Mentype® **Argus X-12**	400	Reactions	Cat. No.	43-13110-0400

Storage

Store all components at –20°C and avoid repeated thawing and freezing. Primer mix and allelic ladder must be stored protected from light. The DNA samples and post-PCR reagents (allelic ladder and DNA Size Standard) should be stored separately from the PCR reagents. The expiry date is indicated on the kit cover.

Additionally required reagents

Additional reagents are needed in order to use the Biotype® PCR Amplification Kit:

Reagent	**Supplier**	**Order number**
JumpStart™ Taq DNA Polymerase hot start, 2.5 U/µL, 50 U or 250 U	Sigma-Aldrich	D4184
Hi-Di™ Formamide, 25 mL	Applied Biosystems	4311320
Matrix Standards BT5 single-capillary instruments (25µl)	Biotype AG	00-10411-0025
Matrix Standards BT5 ABI multi-capillary instruments (25µl)	Biotype AG	00-10421-0025
Matrix Standards BT5 multi-capillary instruments (50µl)	Biotype AG	00-10421-0050

Warnings and safety instructions

The PCR Amplification Kit contains the following potentially hazardous chemicals:

Kit component	Chemical	Hazards
Primer mix, reaction mix and allelic ladder	Sodium azide NaN_3	Very toxic if swallowed, develops toxic gases when it gets in contact with acids

Observe the Material Safety Data Sheets (MSDS) for all Biotype® products, which are available on request. Please contact the respective manufacturers for copies of the MSDS for any additionally needed reagents.

Quality assurance

All kit components undergo an intensive quality assurance process at Biotype AG. The quality of the test kits is permanently monitored in order to ensure unrestricted usability. If you have any questions regarding quality assurance, please do not hesitate to contact us.

Trademarks and patents

Mentype® is a registered trademark of Biotype AG.
GenoProof® is a registered trademark of Qualitype AG.
JumpStart™ is a registered trademark of Sigma-Aldrich.
ABI PRISM®, GeneScan®, Genotyper®, GeneMapper™ and Applied Biosystems are registered trademarks of Applied Biosystems Inc. or its subsidiaries in the U.S. and certain other countries.
6-FAM, POP-4 and Hi-Di are trademarks of Applied Biosystems Inc.
GeneAmp® is a registered trademark of Roche Molecular Systems.
The PCR is covered by patents. Patentees are Hoffmann-La Roche Inc. and F. Hoffmann-La Roche (Roche).
GenBank® is a trademark of National Institute of Health.

Protocols for PCR amplification, electrophoresis and analysis

2. PCR amplification

2.1 Master mix preparation

The table below shows the volumes of all PCR reagents per 25 µL reaction volume, including a sample volume of 1.0 µL (template DNA). The number of reactions to be set up shall be determined taking into account positive and negative control reactions. Add one or two reactions to this number to compensate the pipetting error.

Component	Volume
Nuclease-free water	15.9 µL
Reaction mix **A***	5.0 µL
Primer mix	2.5 µL
Taq DNA Polymerase (hot start, 2.5 U/µL)	0.6 µL
Volume of master mix	24.0 µL

* contains Mg^{2+}, dNTPs, BSA

All components should be mixed (vortex) and centrifuged for about 10 s before preparing the master mix. The DNA volume applied to the assay depends on its concentration. A volume of up to 5 µL may be necessary for DNA trace templates. DNA volumes of more than 5 µL are not recommended, because potential PCR inhibitors may interfere with the process. Fill up the final reaction volume to 25 µL with nuclease-free water.

Generally, DNA templates shall be stored in nuclease-free water or in diluted TE buffer (10 mM Tris HCl, pH 8.0 and 1 mM EDTA), e.g. 0.1x TE buffer.

The primer mixes are adjusted for balanced peak heights at **30 PCR cycles** and **0.35 ng Control DNA XX28** in a reaction volume of 25 µL. If more DNA template is introduced, higher peaks can be expected for small PCR fragments and relatively low peaks for large fragments. Reduce the amount of DNA template to correct this imbalance.

Positive control

For the positive amplification control, dilute the Control DNA XX28 to 0.35 ng in the appropriate volume. Instead of the template DNA, pipette the diluted Control DNA into a reaction tube containing the PCR master mix.

Negative control

For the negative amplification control, pipette nuclease-free water instead of template DNA into a reaction tube which contains the PCR master mix.

Template DNA

Sometimes, the measured value of the DNA concentration varies depending on the used quantification method, so that it may necessary to adjust the optimal DNA amount.

2.2 PCR amplification parameter

Perform a “hot start” PCR in order to activate the Taq DNA Polymerase and to prevent the formation of non-specific amplification products.

The number of cycles depends on the amount of DNA. 30 cycles are recommended for all samples. For critical stains (< 100 pg DNA), it is recommended to increase the number of PCR cycles to 32 cycles, i.e. from 25 to 27 in the second PCR step.

Standard method

Recommended for all DNA samples

Temperature	**Time**	
94°C	4 min (hot start for activation of the JumpStart™ Taq DNA Polymerase)	
96°C	30 s	
63°C	120 s	**5 cycles**
72°C	75 s	
94°C	30 s	
60°C	120 s	**25 cycles**
72°C	75 s	
68°C	60 min	
10°C	∞	hold

Optional

Recommended for stains with small amounts of DNA

Temperature	**Time**	
94°C	4 min (hot start for activation of the JumpStart™ Taq DNA Polymerase)	
96°C	30 s	
63°C	120 s	**5 cycles**
72°C	75 s	
94°C	30 s	
60°C	120 s	**27 cycles**
72°C	75 s	
68°C	60 min	
10°C	∞	hold

Too small amounts of DNA may result in allelic dropouts and imbalances of the peaks. Furthermore, unspecific amplification products could appear. With increasing numbers of cycles, there is the risk of cross contamination caused by minimal amounts of impurities.

3. Electrophoresis using the ABI PRISM® 310 Genetic Analyzer

For general instructions on instrument setup, matrix generation and application of the GeneScan® or GeneMapper™ ID software, refer to the *ABI PRISM® 310 Genetic Analyzer User's Manual*. Electrophoresis using the GeneScan® software is described below.

The virtual **filter set G5** shall be used for combined application of the five fluorescent labels **6-FAM**, **BTG**, **BTY**, **BTR**, and **BTO** (the matrix standard will be called **BT5** hereinafter).

Material

Capillary	47 cm / 50 µm (green)
Polymer	POP-4 for 310 Genetic Analyzer
Buffer	10x Genetic Analyzer Buffer with EDTA

3.1 Matrix generation

Prior to conducting DNA fragment size analysis with the filter set G5, a matrix with the five fluorescent labels 6-FAM, BTG, BTY, BTR, and BTO must be generated.

Colour	**Matrix standard**
Blue (B)	6-FAM
Green (G)	BTG
Yellow (Y)	BTY
Red (R)	BTR
Orange (O)	BTO

Five electrophoresis runs shall be conducted, one for each fluorescent label, 6-FAM, BTG, BTY, BTR, and BTO, under the same conditions as for the samples and allelic ladders of the Biotype® test kit to generate suitable matrix files.

Matrix sample	**Component**	**Volume**
Matrix sample 1	Hi-Di™ Formamide	12.0 µL
	Matrix standard **6-FAM**	1.0 µL
Matrix sample 2	Hi-Di™ Formamide	12.0 µL
	Matrix standard **BTG**	1.0 µL
Matrix sample 3	Hi-Di™ Formamide	12.0 µL
	Matrix standard **BTY**	1.0 µL
Matrix sample 4	Hi-Di™ Formamide	12.0 µL
	Matrix standard **BTR**	1.0 µL
Matrix sample 5	Hi-Di™ Formamide	12.0 µL
	Matrix standard **BTO**	1.0 µL

- Denaturation for 3 min at 95°C
- Cool down to 4°C
- For analysis: load the samples on the tray

- Create a **Sample Sheet** and enter a sample designation

Injection list for matrix generation

Parameter	Set up
Module File	GS STR POP-4 (1 mL) **G5**
Matrix File	**NONE**
Size Standard*	**NONE**
Injection [s]	5
Injection [kV]	15.0
Run [kV]	15.0
Run [°C]	60
Run Time [min]	24

* Prepare matrix standards always **without DNA Size Standard (BTO)**

Analysis of the matrix samples

- Run the GeneScan® software
- **File** → **New** → **Project** (open folder of current run) → **Add Sample Files**
- Select a matrix sample in the **Sample File** column
- **Sample** → **Raw Data**
- Check the matrix samples regarding a flat baseline. As shown in the figure below, there should be at least five peaks with peak heights about 1000-4000 (Y-axis) for each matrix sample (optimal range: 2000-4000)

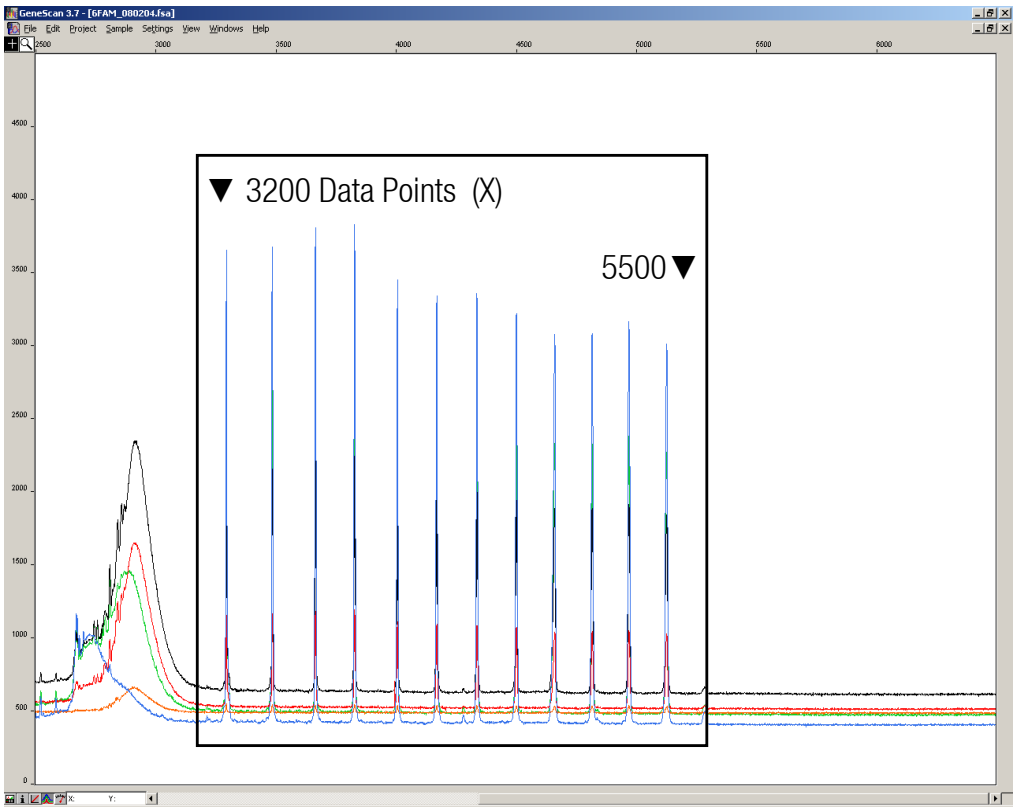

Fig. 2 Electropherogram with raw data of the matrix standard 6-FAM

- Select an analysis range with flat baseline and re-inject the matrix sample if necessary
- Note down start and end value (data points) of the analysis range, e.g. start value 3200, end value 5500
- Calculate the difference, e.g. 5500-3200 = 2300 data points

Generation of a new matrix

- **File** → **New** → **Matrix**

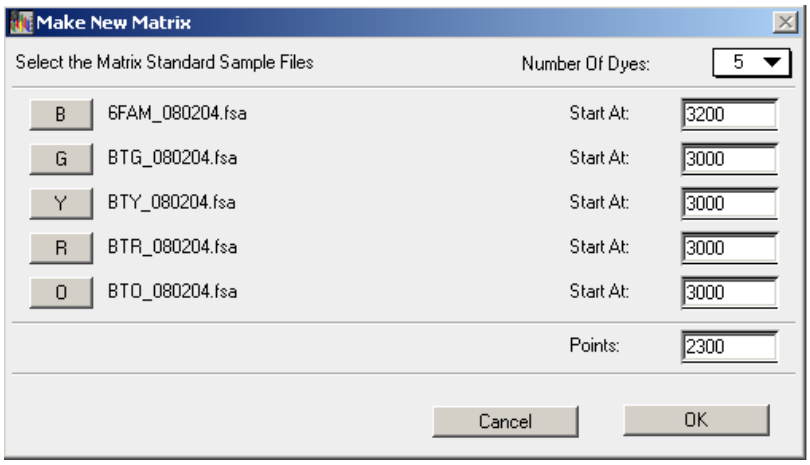

Fig. 3 Matrix sample selection

- Import matrix samples for all dyes (B, G, Y, R, O)
- Enter a **Start At** value, e.g. 3200
- Enter the calculated difference under **Points**, e.g. 2300
- Click on **OK** to calculate the new matrix

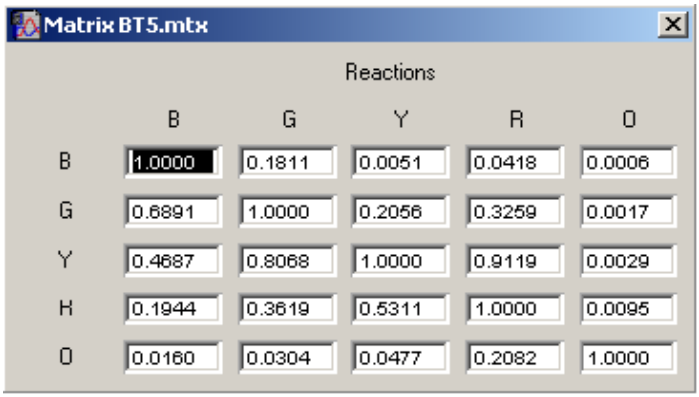

Fig. 4 New matrix BT5

- Save the matrix in the matrix folder: **File** → **Save as**, e.g. Matrix BT5

Matrix check

Check the new matrix with current samples.
- File → **New** → **Project** (open folder of the respective run) → **Add Sample Files**
- Select sample(s) in the **Sample File** column
- Sample → **Install New Matrix** (open matrix folder and select new matrix)
- Re-analyse your samples

There should be **no** pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix.

3.2 Sample preparation

Component	Volume
Hi-Di™ Formamide	12.0 µL
DNA Size Standard 550 (BTO)	0.5 µL
Prepare 12 µL of the mix (formamide + DNA size standard) for all samples	
Add 1 µL PCR product (diluted if necessary) or allelic ladder	

- Denaturation for 3 min at 95°C
- Cool down to 4°C
- For analysis: load the samples on the tray

Signal intensities

Options to increase the signal intensity:
- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

3.3 Setting up the GeneScan® software

- Create a **Sample Sheet** and enter a sample designation

Injection list

Parameter	Set up
Module File	GS STR POP-4 (1 mL) **G5**
Matrix File	e.g. Matrix BT5
Size Standard	e.g. SST-BTO_60-400bp
Injection [s]*	5
Injection [kV]	15.0
Run [kV]	15.0
Run [°C]	60
Run Time [min]**	**26**

* Deviating from the standard settings, the injection time may range between 1 and 10 s depending on the type of sample. If blood samples with very high signal intensities are recorded, a shorter injection time may be selected. For samples with low DNA content an injection time up to 10 s may be necessary.

** Depending on the analysis conditions the run time for Mentype® **Argus X-12** was modified in order to be able to analyse fragments with lengths of up to **400 bp**.

3.4 Analysis parameter

The recommended analysis parameters are:

Analysis Range	Start: 2000 Stop: 10000
Data Processing	Baseline: Checked Multicomponent: Checked Smooth Options: Light
Peak Detection	Peak Amplitude Thresholds B:* Y:* G:* R:* O:* Min. Peak Half Width: 2 pts Polynominal Degree: 3 Peak Window Size: 11 pts**
Size Call Range	Min: 60 Max: 550
Size Calling Method	Local Southern Method
Split Peak Correction	None

* The peak amplitude threshold (cutoff value) corresponds to the minimum peak height that will be detected by the GeneScan® or GeneMapper™ ID software. Thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times as high as the background noise of the baseline.

** Point alleles (i.e. alleles with at least 1 bp difference to the next integer allele) may occasionally not be distinguished. For improved peak detection, minimise the Peak Window Size further.

4. Electrophoresis using the ABI PRISM® 3100-Avant/3100 Genetic Analyzer

For detailed instructions on instrument setup, spectral calibration, or application of the ABI PRISM® 3100 Data Collection software version 1.01 or 1.1 and the GeneScan® software, refer to the *ABI PRISM® 3100-Avant/3100 Genetic Analyzer User's Manual*.

The system with 4 capillaries is named ABI 3100-Avant, and the system with 16 capillaries is named ABI 3100.

The virtual **filter set G5** shall be used for combined application of the five fluorescent labels **6-FAM**, **BTG**, **BTY**, **BTR**, and **BT0** (the matrix standard will be called **BT5** hereinafter).

Material

Capillary	36 cm Capillary Array for 3100-Avant/3100
Polymer	POP-4 Polymer for 3100
Buffer	10x Genetic Analyzer Buffer with EDTA

4.1 Spectral calibration / matrix generation

Proper spectral calibration is critical to evaluate multicolour systems with the ABI PRISM® 3100-Avant/3100 Genetic Analyzer and shall be done prior to conducting fragment length analysis. The calibration procedure creates a matrix which is used to correct the overlapping of fluorescence emission spectra of the dyes.

Spectral calibration comprises the following steps:
- Preparation of the spectral calibration standards
- Loading the standards to the 96-well reaction plate (one sample per capillary)
- Entering the plate composition
- Performing a spectral calibration run and checking the matrix

Setting up the spectral calibration standards

Example for 4 capillaries/ABI 3100-Avant

Component	**Volume**
Hi-Di™ Formamide	60.0 µL
Matrix standard **BT5**	5.0 µL

- Load 12 µL of the mix to a 96-well reaction plate, e.g. position **A1-D1**
- Denaturation for 3 min at 95°C
- Cool down to 4°C

Example for 16 capillaries/ABI 3100

Component	**Volume**
Hi-Di™ Formamide	204.0 µL
Matrix standard **BT5**	17.0 µL

- Load 12 µL of the mix to a 96-well reaction plate, e.g. position **A1-H1** and **A2-H2**
- Denaturation for 3 min at 95°C
- Cool down to 4°C

Performing a spectral calibration run

First of all, the parameter file for **DyeSetG5** must be modified once to achieve successful calibration with the Data Collection software version 1.0.1 or 1.1.

Spectral parameter

To change settings in the parameter file go to the following path:
D:\AppliedBio\Support Files\Data Collection Support Files\CalibrationData\Spectral Calibration\ParamFiles

- Select **MtxStd{Genescan_SetG5}** to open the PAR-file
- Change **Condition Bounds Range** to [1.0; 20.0]. If calibration was not successful, also change **Sensitivity** to 0.1 and **Quality** to 0.8 in a second step
- Select **File** → **Save As** to save the parameter file under a new name, e.g. MtxStd{Genescan_SetG5_Biotype}.par

Always use this parameter file for spectral calibration runs using Biotype® matrix standards **BT5**.

Plate Editor for spectral calibration (I)

- Place the 96-well plate on the autosampler tray
- Run the ABI PRISM® 3100 Data Collection software
- In **Plate View** click **New** to open the **Plate Editor** dialog box
- Enter a name of the plate
- Select **Spectral Calibration**
- Select **96-Well** as plate type and click on **Finish**

Plate Editor for spectral calibration (II)

Parameter	**Set up**
Sample Name	Enter name for the matrix samples
Dye Set	G5
Spectral Run Module	*Default* (e.g. Spect36_POP4)
Spectral Parameters	MtxStd{GeneScan_SetG5_Biotype}.par (parameters created before)

- Click into the column header to select the entire column, select **Edit** → **Fill Down** to apply the information of the selected samples and confirm with **OK**
- Link your reaction plate on the autosampler tray with the created plate ID and start run
- On completion of the run check in the **Spectral Calibration Result** dialog box if all capillaries have successfully passed calibration (label **A**). If individual capillaries are labelled **X**, refer to *ABI PRISM® Genetic Analyzer User's Manual*.
- Click on **OK** to confirm completion of the run

Matrix check

- Select **Tools → Display Spectral Calibration → Dye Set → G5** to review the spectral calibration profile for each capillary
- The quality value (**Q value**) must be greater than 0.95 and the condition number (**C-value**) must be between 1 and 20. Both values must be within the previously determined range
- Check the matrix samples for a flat baseline. There should be five peaks with peak heights of about 1000-5000 (Y-axis) in each matrix sample (optimal range: 2000-4000)
- Check the new matrix with your current samples. There should be **no** pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix
- If calibration was not successful, try to change the **Sensitivity** and **Quality** values in the parameter file as described above
- If all capillaries have passed the calibration, the last calibration file for **Dye Set G5** must be activated manually under **Tools → Set Active Spectral Calibration**. Rename the calibration file under **Set Matrix Name** (e.g. BT5_Date of calibration)

4.2 Sample preparation

Component	**Volume**
Hi-Di™ Formamide	12.0 µL
DNA Size Standard 550 (BTO)	0.5 µL
Prepare 12 µL of the mix (formamide + DNA size standard) for all samples	
Add 1 µL PCR product (diluted if necessary) or allelic ladder	

- Denaturation for 3 min at 95°C
- Cool down to 4°C
- For analysis: load the samples on the tray

Since injections take place simultaneously on all capillaries, 4 or 16 samples must be pipetted on the plate of multi-capillary analysers. If fewer samples are analysed, the empty positions must be filled with 12 µL Hi-Di™ Formamide.

To ensure a reliable allelic assignment on multi-capillary analysers, a number of allelic ladders should be run, independently from sample number.

Room temperature may influence the performance of PCR products on multi-capillary instruments, so that shoulder peaks or split peaks occur especially at low temperatures. Pay attention to keeping ambient conditions as recommended by the instrument manufacturer.

Signal intensities

Options to increase the signal intensity:
- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

4.3 Setting up the GeneScan® software

Edit the default run module in **Dye Set G5** once for the first run.
- Select **Module Editor** to open the dialog box
- Select the appropriate **Run Module** as template from the **GeneScan** table
- Modify the **Injection Voltage** to 3 kV and the **Injection Time** to 10 s

Run Module 3kV_10s_400bp

Parameter	**Set up**
Run Temperature [°C]	*Default*
Cap Fill Volume	*Default*
Maximum Current [A]	*Default*
Current Tolerance [A]	*Default*
Run Current [A]	*Default*
Voltage Tolerance [kV]	*Default*
Pre Run Voltage [kV]	*Default*
Pre Run Time [s]	*Default*
Injection Voltage [kV]	**3.0**
Injection Time [s]*	**10**
Run Voltage [kV]	*Default*
Number of Steps	*Default*
Voltage Step Interval	*Default*
Data Delay Time [s]	*Default*
Run Time [min]**	**20**

***** Deviating from the standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If samples with very high signal intensities are recorded, a shorter injection time may be selected. For samples with low DNA content an injection time of up to 20 s may be necessary.

** Depending on the analysis conditions the run time for Mentype® **Argus X-12** was modified in order to be able to analyse fragments with lengths of up to **400 bp**.

- Click on **Save As**, enter the name of the new module (e.g. 3kV_10s_400bp) and confirm with **OK**
- Click on **Close** to exit the **Run Module Editor**

Starting the run

- Place the prepared 96-well plate on the autosampler tray
- Run the ABI PRISM® 3100 Data Collection software
- In **Plate View** click on **New** to open the **Plate Editor** dialog box
- Enter a name of the plate
- Select **GeneScan**
- Select **96-Well** as plate type and click on **Finish**

Plate Editor

Parameter	**Set up**
Sample Name	Enter name for the samples
Dyes	O
Colour Info	Ladder or sample
Project Name	e.g. 3100_Project1
Dye Set	G5
Run Module*	3kV_10s_400bp
Analysis Module 1	DefaultAnalysis.gsp

* parameter see above

- Complete the table in the **Plate Editor** and click on **OK**
- Click into the column header to select the entire column and select **Edit** → **Fill Down** to apply the information of the selected samples
- Link your reaction plate on the autosampler tray with the created plate ID and start the run
- On completion of the run, view data as **Color Data** in **Array View** of the 3100 Data Collection software or as **Analyzed Sample Files** under D:/AppliedBio/3100/DataExtractor/ExtractRuns

4.4 Analysis parameter

The recommended analysis parameters are:

Analysis Range	Start: 2000 Stop: 10000
Data Processing	Baseline: Checked Multicomponent: Checked Smooth Options: Light
Peak Detection	Peak Amplitude Thresholds B:* Y:* G:* R:* O:* Min. Peak Half Width: 2 pts Polynominal Degree: 3 Peak Window Size: 11 pts**
Size Call Range	Min: 60 Max: 550
Size Calling Method	Local Southern Method
Split Peak Correction	None

* The peak amplitude threshold (cutoff value) corresponds to the minimum peak height that will be detected by the GeneScan® or GeneMapper™ ID software. Thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times as high as the background noise of the baseline.

** Point alleles (i.e. alleles with at least 1 bp difference to the next integer allele) may occasionally not be distinguished. For improved peak detection, minimise the Peak Window Size further.

5. Electrophoresis using the ABI PRISM® 3130/3130xl Genetic Analyzer

For detailed instructions on instrument setup, spectral calibration, or application of the ABI PRISM® Data Collection software version 3.0 and the GeneMapper™ ID software, refer to the *ABI PRISM ® 3130/3130xl Genetic Analyzers Getting Started Guide*.

The system with 4 capillaries is named ABI 3130, and the system with 16 capillaries is named ABI 3130xl.

The virtual **filter set Any5Dye** shall be used for the combined application of the five fluorescent labels **6-FAM**, **BTG**, **BTY**, **BTR** and **BT0** (the matrix standard will be called **BT5** hereinafter).

Material

Capillary	36 cm Capillary Array for 3130/3130xl
Polymer	POP-4 Polymer for 3130
Buffer	10x Genetic Analyzer Buffer with EDTA

5.1 Spectral calibration / matrix generation

Prior to conducting DNA fragment size analysis, it is necessary to perform a spectral calibration with the five fluorescent labels 6-FAM, BTG, BTY, BTR, and BTO for each analyzer. The calibration procedure creates a matrix which is used to correct the overlapping of fluorescence emission spectra of the dyes.

Spectral calibration comprises the following steps:
- Preparation the spectral calibration standards
- Loading the standards to the 96-well reaction plate (one sample per capillary)
- Creating the instrument protocol for spectral calibration (Protocol Manager)
- Defining the plate composition in the plate editor (Plate Manager)
- Performing a spectral calibration run and checking the matrix

Setting up the spectral calibration standards

Example for 4 capillaries/ABI 3130

Component	**Volume**
Hi-Di™ Formamide	60.0 µL
Matrix standard **BT5**	5.0 µL

- Load 12 µL of the mix to a 96-well reaction plate, e.g. position **A1-D1**
- Denaturation for 3 min at 95°C
- Cool down to 4°C

Example for 16 capillaries/ABI 3130xl

Component	**Volume**
Hi-Di™ Formamide	204.0 µL
Matrix standard **BT5**	17.0 µL

- Load 12 µL of the mix to a 96-well reaction plate, e.g. position **A1-H1** and **A2-H2**
- Denaturation for 3 min at 95°C
- Cool down to 4°C

Performing a spectral calibration run

- Place the 96-well plate on the autosampler tray
- In the **Protocol Manager** of the Data Collection software click on **New** in **Instrument Protocol** to open the **Protocol Editor** dialog box

Instrument Protocol for spectral calibration

Protocol Editor	**Set up**
Name	*User* (e.g. Spectral36_POP4_BT5)
Type	SPECTRAL
Dye Set	Any5Dye
Polymer*	*User* (e.g. POP4)
Array Length*	*User* (e.g. 36cm)
Chemistry	Matrix Standard
Run Module*	*Default* (e.g. Spect36_POP4_1)

* Depends on the type of polymer and length of capillary used

- Click on **OK** to leave the **Protocol Editor** dialog box
- In the **Plate Manager** of the Data Collection software, click on **New** to open the **New Plate Dialog** box

Plate Editor for spectral calibration (I)

New Plate Dialog	**Set up**
Name	e.g. Spectral_BT5_date
Application	Spectral Calibration
Plate Type	96-Well
Owner Name / Operator Name	…

- Click on **OK**. A new table in the **Plate Editor** will open automatically

Plate Editor for spectral calibration (II)

Parameter	**Set up**
Sample Name	Enter name for the matrix samples
Priority	e.g. 100
Instrument Protocol 1	Spectral36_POP4_BT5 (setting described before)

- Click into the column header to select the entire column, select **Edit** → **Fill Down** to apply the information to all selected samples, and click on **OK**
- In the **Run Scheduler** click on **Find All**, select **Link** to link the reaction plate on the autosampler up with the newly created plate record (position A or B) and start the run

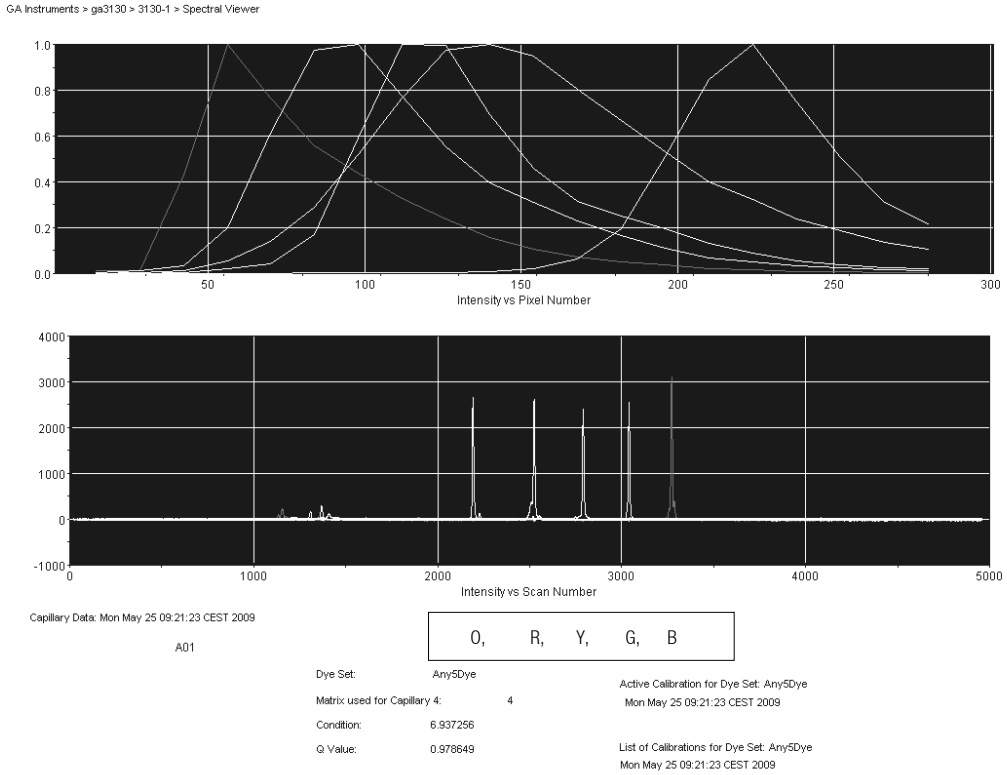

Fig. 5 Electropherogram of spectral calibration with matrix standard BT5 on an ABI 3130

Matrix check

- The quality value (**Q value**) of each capillary must be greater than 0.95 and the condition number range (**C value**) must be between 1 and 20.
- Check the matrix samples for a flat baseline. As shown in the figure above, there should be five peaks with peak heights of about 1000-5000 (Y-axis) in each matrix sample (optimal range: 2000-4000)
- Check the new matrix with your current samples. There should be **no** pull-up peaks between the dye panels (B, G, Y, R, O) with the new matrix
- If calibration was not successful, use the optimised values and repeat the calibration run
- If all capillaries have passed the test, the last calibration file for the Dye Set **Any5Dye** is activated automatically in the **Spectral Viewer**. **Rename** the calibration file (e.g. BT5_Date of calibration) using the respective button

5.2 Sample preparation

Component	Volume
Hi-Di™ Formamide	12.0 µL
DNA Size Standard 550 (BTO)	0.5 µL
Prepare 12 µL of the mix (formamide + DNA size standard) for all samples	
Add 1 µL PCR product (diluted if necessary) or allelic ladder	

- Denaturation for 3 min at 95°C
- Cool down to 4°C
- For analysis: load the samples on the tray

Since injections take place simultaneously on all capillaries, 4 or 16 samples must be pipetted on the plate of multi-capillary analysers. If fewer samples are analysed, the empty positions must be filled with 12 µL Hi-Di™ Formamide.

To ensure a reliable allelic assignment on multi-capillary analysers, a number of allelic ladders should be run, independently from sample number.

Room temperature may influence the performance of PCR products on multi-capillary instruments, so that shoulder peaks or split peaks occur especially at low temperatures. Pay attention to keeping ambient conditions as recommended by the instrument manufacturer.

Signal intensities

Options to increase the signal intensity:
- Reduce the volume of the DNA Size Standard 550 (BTO) to peak heights of about 500 relative fluorescent units (RFU)
- Purify the PCR products before starting the analysis

5.3 Setting up the GeneMapper™ ID software

Edit the run module as follows for the first run:

- In the **Module Manager** of the Data Collection software click on **New** to open the **Run Module Editor** dialog box

Run Module 3kV_10s_400bp

Paramater	**Set up**
Oven Temperature [°C]	*Default*
Poly Fill Volume	*Default*
Current Stability [µA]	*Default*
PreRun Voltage [kV]	*Default*
PreRun Time [s]	*Default*
Injection Voltage [kV]	**3.0**
Injection Time [s]*	**10**
Voltage Number of Steps	*Default*
Voltage Step Interval	*Default*
Data Delay Time [s]	*Default*
Run Voltage [kV]	*Default*
Run Time [s]**	**1200**

* Deviating from the standard settings, the injection time may range between 1 and 20 s depending on the type of sample. If samples with very high signal intensities are recorded, a shorter injection time may be selected. For samples with low DNA content an injection time of up to 20 s may be necessary.

** Depending on the analysis conditions the run time for Mentype® **Argus X-12** was modified in order to be able to analyse fragments with lengths of up to **400 bp**.

- Click on **Save As**, enter the name of the new module (e.g. 3kV_10s_400bp) and confirm with **OK**
- Click on **Close** to exit the **Run Module Editor**

Starting the run

- Place the prepared 96-well plate on the autosampler tray
- In the **Protocol Manager** of the Data Collection software, click on **New** in the **Instrument Protocol** window to open the **Protocol Editor** dialog box

Instrument Protocol

Protocol Editor	**Set up**
Name	Run36_POP4_BT5_20min
Type	REGULAR
Run Module*	3kV_10s_400bp
Dye Set	Any5Dye

* parameter see above

- Click on **OK** to exit the **Protocol Editor**

Prior to each run, it is necessary to create a plate definition as follows:

- In the **Plate Manager** of the Data Collection software click on **New** to open the **New Plate Dialog** box

GeneMapper™ Plate Editor (I)

New Plate Dialog	**Set up**
Name	e.g. Plate_BT5_Date
Application	Select GeneMapper Application
Plate Type	96-Well
Owner Name / Operator Name	…

- Click on **OK**. A new table in the **Plate Editor** opens automatically

GeneMapper™ Plate Editor (II)

Parameter	**Set up**
Sample Name	Enter a name of the samples
Priority	e.g. 100 (Default)
Sample Type	Sample or allelic ladder
Size Standard	e.g. SST-BTO_60-400bp
Panel	e.g. X12_Panels_v0
Analysis Method	e.g. Analysis_HID_3130
Snp Set	-
User-defined 1-3	-
Results Group 1	(select results group)
Instrument Protocol 1	Run36_POP4_BT5_20min (setting described before)

- Click into the column header to select the entire column, select **Edit → Fill Down** to apply the information to all selected samples and click on **OK**
- In the **Run Scheduler,** click on **Find All**, select **Link** to link the reaction plate on the autosampler up with the newly created plate record (position A or B) and start the run
- During the run, view **Error Status** in the **Event Log** or examine the quality of the raw data for each capillary in the **Capillaries Viewer** or the **Cap/Array Viewer**
- View data as overview in **Run History** or **Cap/Array Viewer** of the Data Collection software. Run data are saved in the **Run Folder** of the previously chosen **Result Group**

5.4 Analysis parameter / analysis method

The recommended settings in the worksheet the Peak Detector are:

Peak Detection Algorithm	Advanced
Ranges	Analysis: Partial Range Start Pt: 2000; Stop Pt: 10000 Sizing: All Sizes
Smoothing and Baselining	Smoothing: Light Baseline Window: 51 pts
Size Calling Method	Local Southern Method
Peak Detection	Peak Amplitude Thresholds B:* Y:* G:* R:* O:* Min. Peak Half Width: 2 pts Polynominal Degree: 3 Peak Window Size: 11 pts** Slope Thresholds: 0.0

* The peak amplitude threshold (cutoff value) corresponds to the minimum peak height that will be detected by the GeneMapper™ ID software. The thresholds are usually 50-200 RFU and should be determined individually by the laboratory. Recommendation: The minimal peak height should be three times as high as the background noise of the baseline.

** Point alleles (i.e. alleles with at least 1 bp difference to the next integer allele) may occasionally not be distinguished. For improved peak detection, minimise the Peak Window Size further.

6. Analysis

For general instructions on automatic sample analysis, refer to the *GeneScan®* or *GeneMapper™ ID Software User's Manual.*

Finding the exact lengths of the amplified products depends on the device type, the conditions of electrophoresis, as well as the DNA size standard used. Due to the complexity of some loci, determining the size should be based on evenly distributed references. The DNA Size Standard 550 (BTO) shall thus be used with the following lengths of fragments **60**, **80**, **90**, **100**, **120**, **140**, **160**, **180**, **200**, **220**, **240**, **250**, **260**, **280**, **300**, **320**, **340**, **360**, **380**, **400**, **425**, **450**, **475**, **500**, **525**, and **550 bp**.

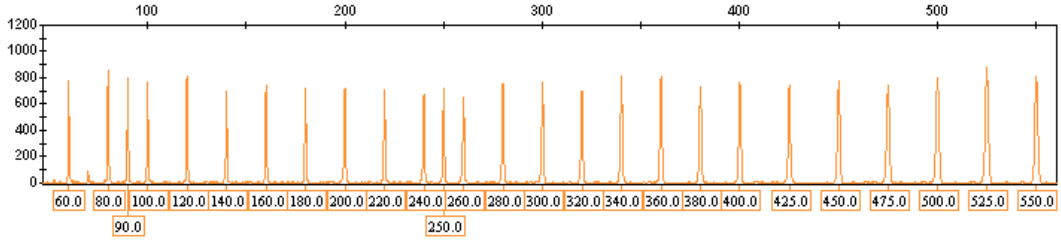

Fig. 6 Electropherogram of the DNA Size Standard 550 (BTO), fragments with lengths in bp

Note: The basic template files for the DNA Size Standard 550 (BTO) has to be adjusted to 400 bp within the GeneMapper™ ID software. The new template could be saved as e.g. SST-BTO_60-400bp and used for further analyses

Special feature

Primer sequences of the Mentype® **Argus X-12** are different to that of the Mentype® **Argus X-8** in order to avoid allelic overlapping. Furthermore the changings result in more robustness and sensitivity. Because of the new primer design for the locus **HPRTB** a dinucleotide-deletion (AG) 48-49 bp downstream of the repeat unit (Mertens et al. 1999, Gomes et al. 2007) will not be detected any more so that just the real repeat units will be shown. If necessary point alleles (like 11.2, 12.2), that were detected by the primers of the Mentype® **Argus X-8** or **Argus X-UL**, should be rounded up the next full allele for comparing the results generated with the Mentype® **Argus X-12** primers.

6.1 Biotype® template files

Allele allocation should be carried out with a suitable analysis software, e.g. GeneMapper™ ID or Genotyper® software in combination with the Mentype® **Argus X-12** template files from Biotype AG. Template files are available from our homepage or as CD-ROM on request.

Recommended Biotype® templates for GeneMapper™ ID software are:

Panels	Biotype_Panels_v2 (choose kit)	or higher versions
BinSets	Biotype_Bins_v2	or higher versions
Size Standard	SST-BTO_60-500bp (adjust up to 400bp, adjustment described before)	
Analysis Method	Analysis_HID_310	
	Analysis_HID_3130	
	Analysis_HID_310_50rfu	
	Analysis_HID_3130_50rfu	
Plot Settings	Plots_Blue	
	Plots_Green	
	Plots_Yellow	
	Plots_Red	
	Plots_4dyes	
Table Settings	Table for 2 alleles	
	Table for 10 alleles	

Panels and BinSets always have to be used whereas the other template files are optional.

Recommended Biotype® template files for Genotyper® software are:

Argus X12_v0	or higher versions

General procedure for the analysis

1. Check the DNA size standard
2. Check the allelic ladder
3. Check the positive control
4. Check the negative control
5. Analyse and interpret the sample data

6.2 Controls

The Control DNA XX28 of the test kit and other commercially available DNA from standard cell lines represent the following alleles:

Table 3. Allele assignment of Mentype® Argus X-12

STR Locus	KontroII-DNA XX28	ATCC K-562	CCR 9947A	CCR 9948	CCR 3657
Amelogenin	X / X	X / X	X / X	X / Y	X / Y
DXS7132	13 / 14	13 / 13	12 / 12	13	12
DXS7423	14 / 15	17 / 17	14 / 15	14	13
DXS8378	10 / 12	10 / 10	10 / 11	11	12
DXS10074	18 / 20	17 / 17	16 / 19	18	7
DXS10079	18 / 21	17 / 17	20 / 23	19	19
DXS10101	28.2 / 28.2	31 / 31	30 / 31	32	29.2
DXS10103	16 / 19	17 / 17	17 / 17	18	20
DXS10134	36 / 38.3	32 / 32	35 / 36	34	34
DXS10135	16 / 30	27 / 27	21.1 / 27	22	25
DXS10146	26 / 46.2	29 / 29	28 / 28	29	27
DXS10148	18 / 23.1	23.1 / 23.1	22.1 / 23.1	23	23.1
HPRTB	12 / 13	13 / 13	14 / 14	14	13

For further confirmation, the table above displays the X-chromosomal alleles of the reference DNA purchased from ATCC (http://atcc.org/Produtcs/PurifiedDNA.cfm#celllines) as well as three reference DNA purchased from Coriell Cell Repositories (CCR; http://locus.umdnj.edu/nigms/) that is up to standard of Szibor et al. (2003c).

6.3 Lengths of fragments and alleles

Table 4 to **table 7** show the fragment lengths of individual alleles that refer to the DNA Size Standard 550 (BTO). All analyses have been performed on an ABI PRISM® 310/3130 Genetic Analyzer with POP-4 polymer. Different analysis instruments, DNA Size Standards or polymers may result in different fragment lengths. In addition, a visual alignment with the allelic ladder is recommended.

Scaling

Horizontal: 75-405 bp
Vertical: Depending on signal intensity

Figure 7

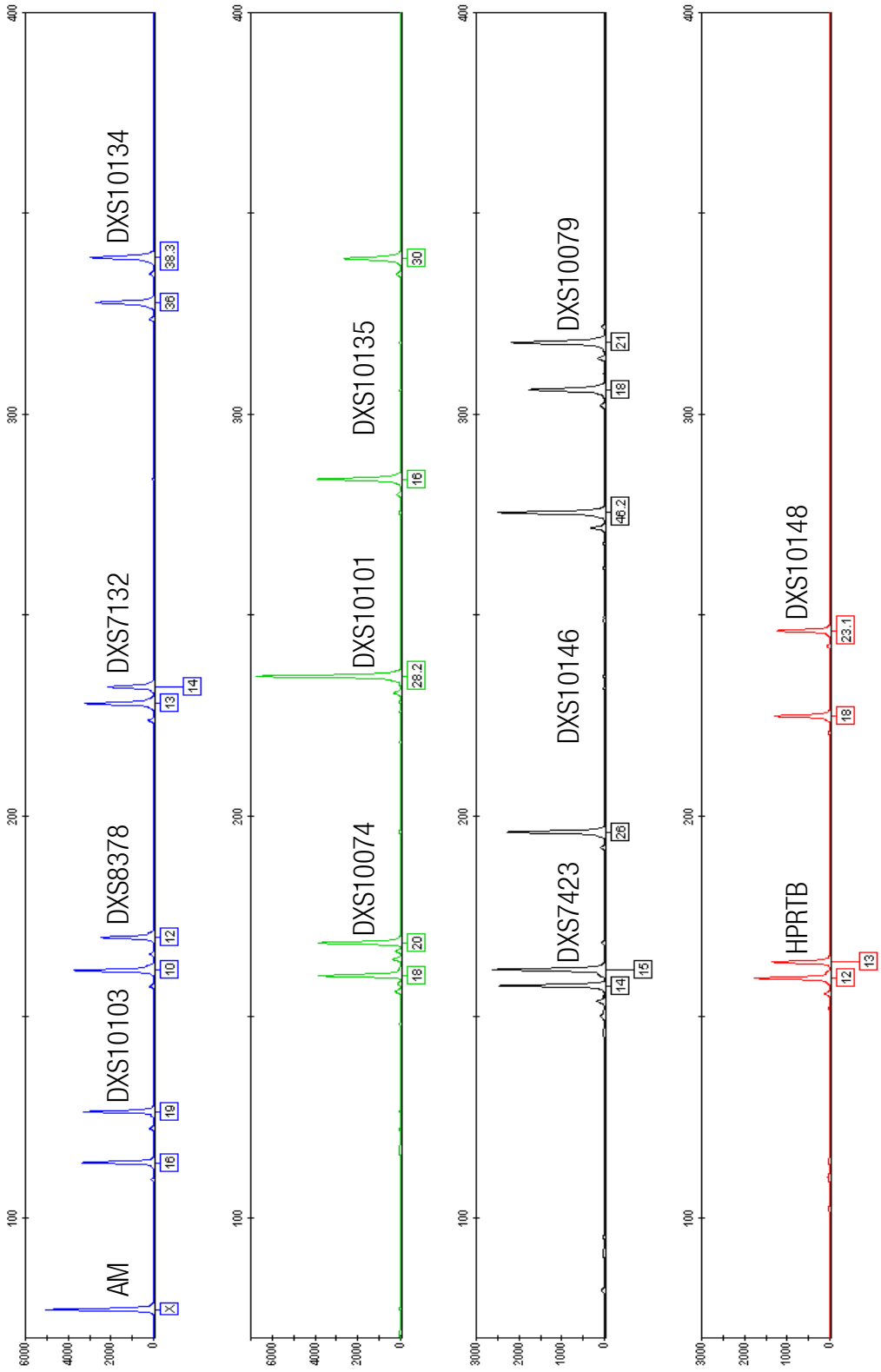

Fig. 7 Electropherogram of Mentype® **Argus X-12** using 350 pg male Control DNA XX28. Analysis was performed on an ABI PRISM® 3130 Genetic Analyzer. Allele assignment was performed using the GeneMapper™ ID software and the Mentype® **Argus X-12** template file.

Figure 8

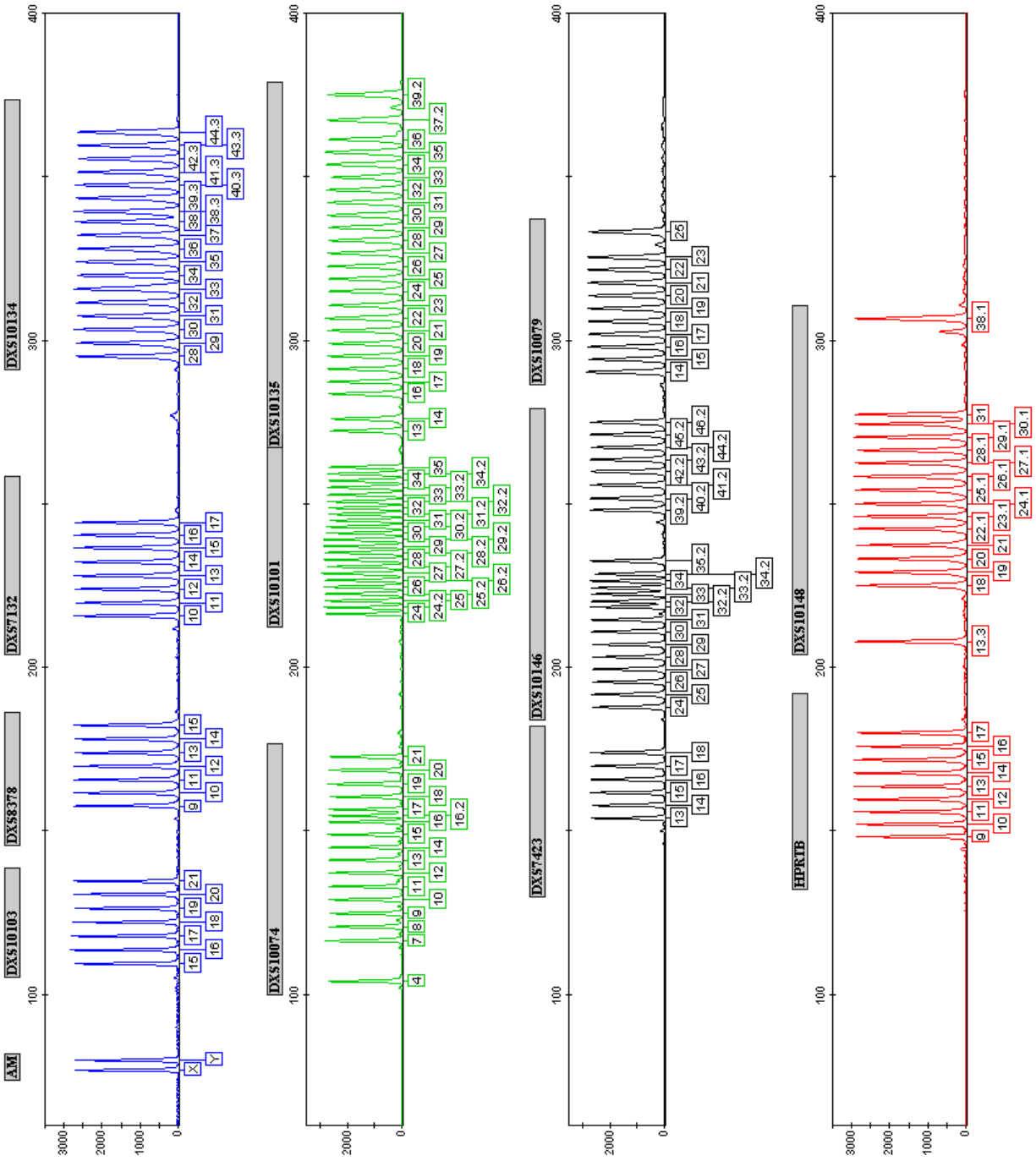

Fig. 8 Electropherogram of the allelic ladder Mentype® **Argus X-12** analysed on an ABI PRISM® 310 Genetic Analyzer. Allele assignment was performed using the GeneMapper™ ID Software and the Mentype® **Argus X-12** template file.

Table 4. Fragment lengths of the allelic ladder Mentype® Argus X-12 analysed on an ABI PRISM® 310/3130 Genetic Analyzer (blue panel)

Marker/Allele	Size [bp]*	Marker/Allele	Size [bp]*	Further alleles**	Marker/Allele	Size [bp]*	Further allele**
Amelogenin	**6-FAM**	**DXS8378**	**6-FAM**		**DXS10134**	**6-FAM**	
X	77	9	158	8	28	295	
Y	80	10	162		29	299	
		11	166		30	303	
DXS10103	**6-FAM**	12	170		31	307	31.1
15	109	13	174		32	311	32.1
16	114	14	178		33	315	33.1
17	118	15	182		34	319	
18	122				35	324	35.3
19	126	**DXS7132**	**6-FAM**		36	328	
20	130	10	216		37	332	37.2, 37.3
21	135	11	220		38	336	38.2
		12	224		38.3	339	
		13	228		39.3	343	39, 39.2
		14	232		40.3	347	40
		15	236		41.3	351	41
		16	240		42.3	355	
		17	244		43.3	359	
					44.3	363	

Table 5. Fragment lengths of the allelic ladder Mentype® Argus X-12 analysed on an ABI PRISM® 310/3130 Genetic Analyzer (green panel)

Marker/Allele	Size [bp]*	Further alleles**	Marker/Allele	Size [bp]*	Further alleles**	Marker/Allele	Size [bp]*	Further alleles**
DXS10074	**BTG**		**DXS10101**	**BTG**		**DXS10135**	**BTG**	
4	104		24	216		13	273	
7	116		24.2	218		14	276	15
8	121		25	220		16	283	16.1
9	125		25.2	222		17	287	17.1
10	129		26	224		18	291	18.1
11	133		26.2	226		19	295	19.1
12	137		27	229		20	299	20.1
13	141		27.2	231		21	303	21.1
14	145	14.3	28	233		22	307	22.1
15	149		28.2	235		23	311	23.1
16	152		29	237		24	315	24.1, 24.2
16.2	154		29.2	239		25	319	25.1
17	156		30	241		26	323	26.1
18	160		30.2	243		27	327	
19	164	19.3	31	245		28	331	28.1
20	168		31.2	247		29	334	29.1
21	172		32	249		30	338	
			32.2	251		31	342	
			33	253		32	346	32.1
			33.2	255		33	348	33.2
			34	257		34	354	34.1
			34.2	259		35	358	35.1, 35.2
			35	261	36	36	362	
						37.2	368	
						39.2	375	

Table 6. Fragment lengths of the allelic ladder Mentype® Argus X-12 analysed on an ABI PRISM® 310/3130 Genetic Analyzer (yellow panel)

Marker/Allele	Size [bp]*	Further alleles**	Marker/Allele	Size [bp]*	Further alleles**	Marker/Allele	Size [bp]*
DXS7423	**BTY**		**DXS10146**	**BTY**		**DXS10079**	**BTY**
13	154	12	24	188		14	290
14	158		25	192		15	294
15	162		26	196		16	298
16	166		27	199	27.3	17	302
17	170		28	203		18	306
18	174		29	207		19	310
			30	211		20	314
			31	215		21	318
			32	219		22	322
			32.2	221		23	326
			33	223		25	333
			33.2	225			
			34	227			
			34.2	229			
			35.2	233			
			39.2	248			
			40.2	252			
			41.2	256			
			42.2	260			
			43.2	264			
			44.2	267			
			45.2	271			
			46.2	275			

Table 7. Fragment lengths of the allelic ladder Mentype® Argus X-12 analysed on an ABI PRISM® 310/3130 Genetic Analyzer (red panel)

Marker/allele	Size [bp]*	Further alleles**	Marker/allele	Size [bp]*	Further alleles**
HPRTB	**BTR**		**DXS10148**	**BTR**	
9	148	8	13.3	207	
10	152		18	224	
11	156		19	228	
12	160		20	232	
13	164		21	236	
14	168		22.1	241	
15	172		23.1	245	23
16	176		24.1	249	24
17	180		25.1	253	
			26.1	257	
			27.1	261	
			28.1	265	
			29.1	269	
			30.1	273	
			31	276	
			38.1	305	

* rounded to integer

** The “off-ladder” alleles of Biotype’s DNA pool are allocated with the actual Biotype® template files for GeneMapper™ ID or Genotyper® software. For further alleles see amongst others http://www.cstl.nist.gov/biotech/strbase/str_fact.htm

7. Interpretation of results

As mentioned above, post PCR analysis and automatic allele assignment with suitable analysis software ensure a precise and reliable discrimination of alleles.

Pull-up peaks

Pull-up peaks may occur if peak heights are outside the linear detection range (>3000 RFU), or if an incorrect matrix was applied. They appear at positions of specific peaks in other colour channels, typically with lower signal intensities. Peak heights should not exceed 3000 RFU in order to prevent pull-up peaks.

Stutter peaks

The occurrence of stutter peaks depends on the sequence of the repeat structure and the number of alleles. n-4 peaks are caused by a loss of a repeat unit during amplification of tetranucleotide STR motives, caused by slippage effects of the Taq DNA Polymerase. Interpretation of those peaks should be done in accordance with the Template Files of the Genotyper® and GeneMapper™ ID software.

Template-independent addition of nucleotides

Because of its terminal transferase activity, the Taq DNA Polymerase tends to add an adenosine radical at the 3'-end of the amplified DNA fragments. The artefact peak is one base shorter than expected (-1 peaks). All Biotype® primers are designed to minimise these artefacts. Artefact formation is further reduced by the final extension step of the PCR protocol at 68°C for 60 minutes. Peak height of the artefact correlates with the amount of DNA. Laboratories should define their own limits for analysis of the peaks.

Artefacts

Room temperature may influence the performance of PCR products on multi-capillary instruments, so that shoulder peaks or split peaks occur. If shoulder or split peaks appear, we recommend injecting the sample again.

8. Usage of the X-chromosomal STRs and their characteristics

In principle, the guidelines of the ISFG (www.isfg.org) are valid for working with X-chromosomal STRs, too. However, the following special characteristics should be pointed out: Typing of X-chromosomal markers is only permissible when the phenotypic and genetic sex is identical. The genetic sex can easily be determined by the analysis of the Amelogenin locus. If the phenotypic sex differs from the genotypic sex, results from ChrX-typing could not be used. Following the national laws, this information has to be handled strictly confidential and should only be forwarded to the analysed person on request.

Generation of X-chromosomal profiles for kinship testing should be taken into account whenever autosomal markers do not give reliable or unequivocal results, mainly in deficiency cases. In some instances, e.g. for complex kinship and paternity testing, markers located on the sex chromosomes may turn out to be more informative than their autosomal counterparts, and the use of sex-chromosomal markers may therefore substantially improve the efficiency of a kinship test. This arises from the fact that the mean exclusion chance (MEC) of a X-chromosomal marker (for calculation see below) is consistently larger than that of an autosomal marker with the same allele frequencies.

Calculation of population-genetic data

The Forensic ChrX Research Group initiated the online data base “ChrX-STR.org” (http://www.chrx-str.org) that calculates population-genetic data on the basis of X-chromosmal allele frequencies (Szibor et al., 2006).

The formula for calculation of the “Polymorphism Information Content” (PIC) was published by Botstein et al. (1980), the one for the “Expected Heterocygosity” (HET) by Nei and Roychoudhury (1974). Both can be used for autosomal or X-chromosomal marker. Krüger et al. (1968) introduced the formula for the “Mean Exclusion Chance“ $MEC_{Krüger}$ which was developed for either autosomal marker or for trios. With exception of deficient cases, $MEC_{Krüger}$ is not suitable for X-chromosomal marker. Here, the paternal grandmother can be analysed instead of the putative father. Kishida et al. (1997) devised the $MEC_{Kishida}$ for X-chromosomal marker in consideration of trios including a daughter. In comparison with $MEC_{Krüger}$, $MEC_{Kishida}$ is more complex which highlights the fact that in trios involving a daughter X-STRs are more efficient then autosomal markers. Finally, Desmarais et al.(1998) introduced formulae for the “Mean Exclusion Chance“ of ChrX markers in trios involving daughters as well as in father-daughter duos without information about the maternal genotype. $MEC_{Desmarais}$ is equivalent to $MEC_{Kishida}$ whereas $MEC_{Desmarais\ Duo}$ can also be used for maternity testing of mother-son duos. The formula for calculation of the “Power of Discrimination” was published by Desmarais et al. (1998).

$$PIC = 1 - \sum_{i=1}^{n} f_i^{\,2} - 2 \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} f_i^{\,2} f_j^{\,2}$$

$$HET = \frac{n}{n-1}\left(1 - \sum_{j=1}^{K} f^2\right)$$

$MEC_{Krüger} = \Sigma_i\, f_i^3\, (1 - f_i)^2 + \Sigma_i\, f_i\, (1 - f_i)^3 + \Sigma_{i<j}\, f_i\, f_j\, (f_i + f_j)\, (1 - f_i - f_j)^2$

$MEC_{Kishida} = \Sigma_i\, f_i^3\, (1 - f_i) + \Sigma_i\, f_i\, (1 - f_i)^2 + \Sigma_{i<j}\, f_i\, f_j\, (f_i + f_j)\, (1 - f_i - f_j)$

$MEC_{Desmarais} = 1 - \Sigma_i\, f_i^2 + \Sigma_i\, f_i^4 - (\Sigma_{i<j}\, f_i^2)^2$

$MEC_{Desmarais\ Duo} = 1 - 2\Sigma_i\, f_i^2 + \Sigma_i\, f_i^3$

$PD_{female} = 1 - 2(\Sigma_i\, f_i^2)^2 + \Sigma_i\, f_i^4$

$PD_{male} = 1 - \Sigma_i\, f_i^2$

All important population-genetic data could be calculated with the GenoProof® software from Qualitype AG.

Linkage groups

In contrast to ealier findings (Szibor 2003c), recent studies showed (Tillmar et al. 2008, Machado 2009) that markers of linkage groups three (HPRTB, DXS10101) and four (DXS10134, DXS7423) cannot be regarded as unlinked. The possibility of linkage between these markers should be considered in calculation and interpretation of relationship tests in deficiency cases. If necessary both probabilities should be calculated from the linkage groups 1,2,3 **and** linkage groups 1,2,4. For use and calculation of X-chromosomal markers see Krawczak M, 2007 and http://linkage.rockefeller.edu.

Kinship tests and paternity cases with ChrX STRs

Note: X-chromosomal markers are particularly informative for inferring parent–offspring relationships that involve at least one female, i.e. mother–daughter, mother–son, and father–daughter duos. For paternity cases only testing between father and daughter is possible, since fathers leave their only X-chromosome to their daughter, and sons get the X-chromosome from their mother.

Deficiency cases

If the putative father (PV) descends from the putative grandmother (PGM) without any doubt, his X-chromosomal characteristics originate from the PGM. Therefore, she (PGM) can be analysed X-chromosomally instead of him (PV). The calculation of the PGM-likelihood with X-chromosomal markers (PGM-daughter) may be performed like the calculation with autosomal markers (PV-daughter). Here, the MEC values according to the formula of Krüger et al., 1968 shall be applied, whereas only unlinked X-STR markers of the Mentype **Argus X-12** can be used. For markers which are in coupling equilibrium, haplotype frequencies must be involved for calculation.

Siblings and distant relationships

The X-chromosomal analysis – without parental samples – can **not** be used as relationship test in the following cases:
- Different putative fathers could be father of the child
- Half-brothers with same father and different mothers
- Brothers and half-brothers with same mother (statistically, they have 50% of the characteristics in common, thus the test is comparable to a test with autosomal markers)

Whenever distant relationship should be analysed, genotyping of X-chromosomal markers is very useful, because the usage of coupled markers allows ChrX-haplotyping. Three markers of each linkage group from Mentype® **Argus X-12** have to be handled as haplotype for genotyping. For further details see Szibor et al., 2003c, for calculation see the online data base “ChrX-STR.org” (http://www.chrx-str.org).

Usage of X-chromosomal markers for forensic traces

In most cases the use of autosomal markers for profiling is much better because all autosomal loci have two alleles. Whenever female DNA traces have to be analysed in a male background, e.g. female traces under nails of a man, X-chromosomal markers are advantaged. The likelihood that one allele of the trace is covered by the male background is only half in comparison to autosomal tests. Note: International DNA databases usually do not include markers of the X-chromosome.

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